Beta amyloid peptide profiling

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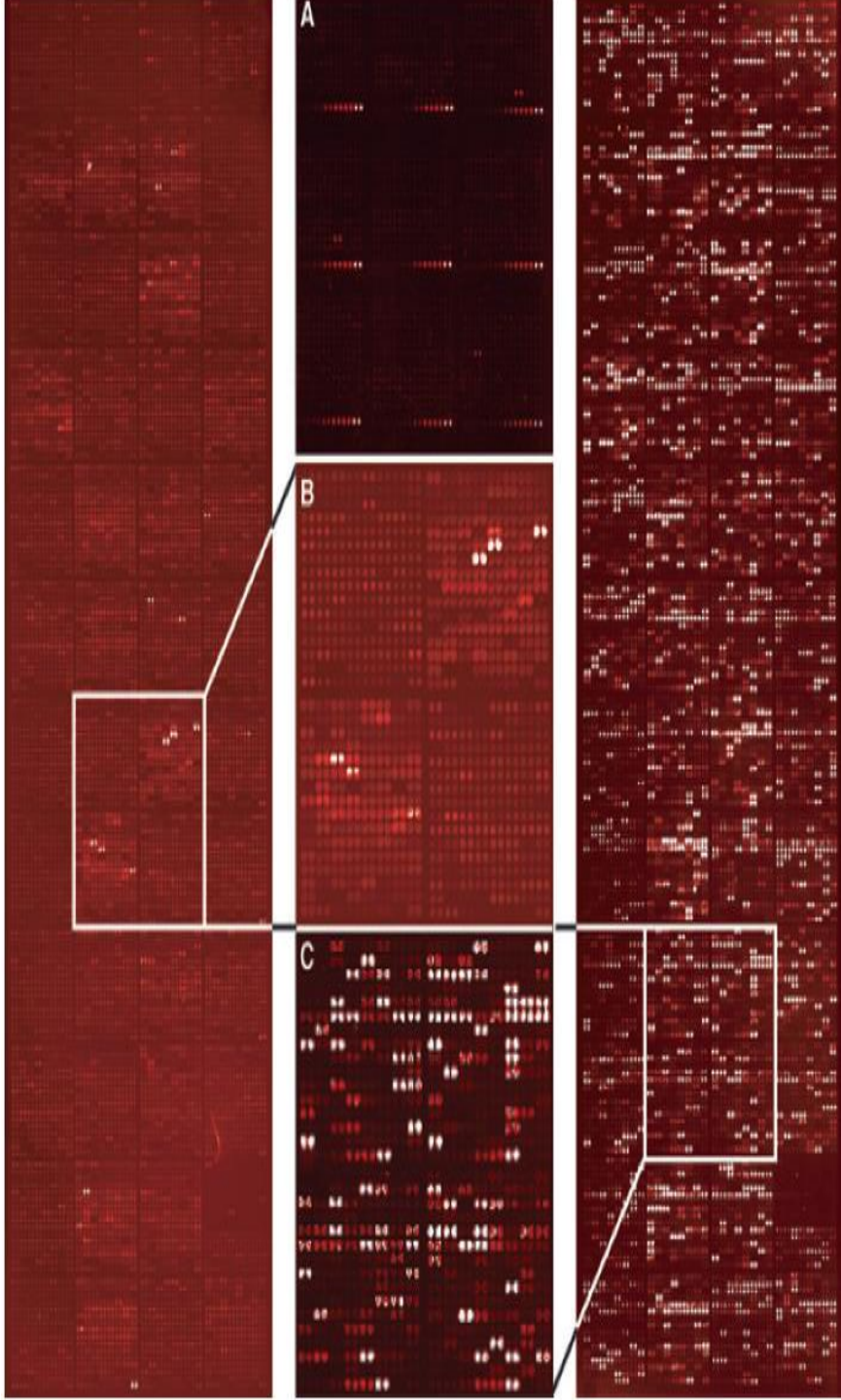
Winter 1397

Content:

- Protein profiling
- 2 Dimensional Electrophoresis
- Mass Spectrometry
  - MALDI TOF
  - SELDI TOF
- Protein micro array
- Beta amyloid peptide

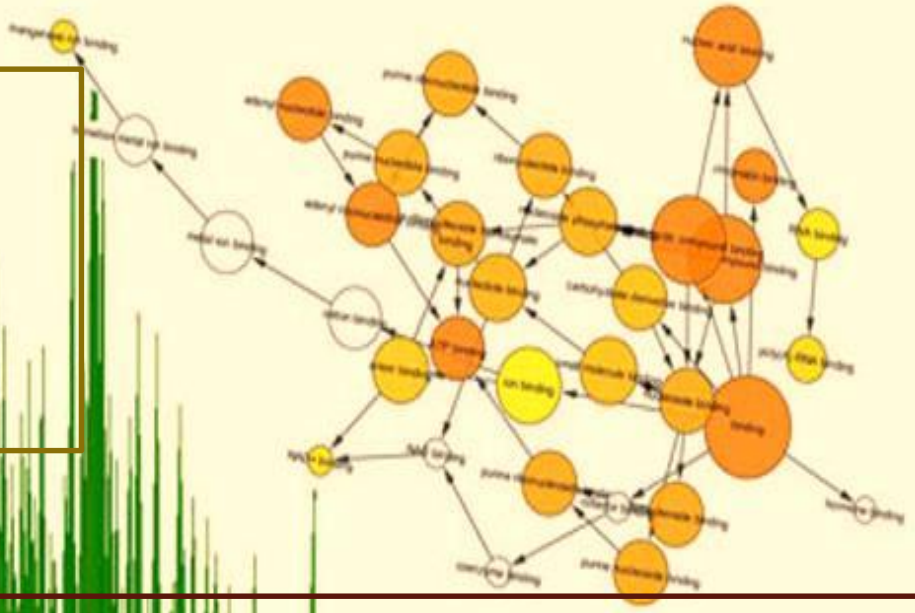


Protein key roles:

Catalysts

Messengers

Transporters



All research related to proteins increase our understanding of their:

Levels

Functions

Regulations

Interactions

Modifications

Localization in cells

Proteomics = Protein + Genomics

Work in proteomics encompasses:

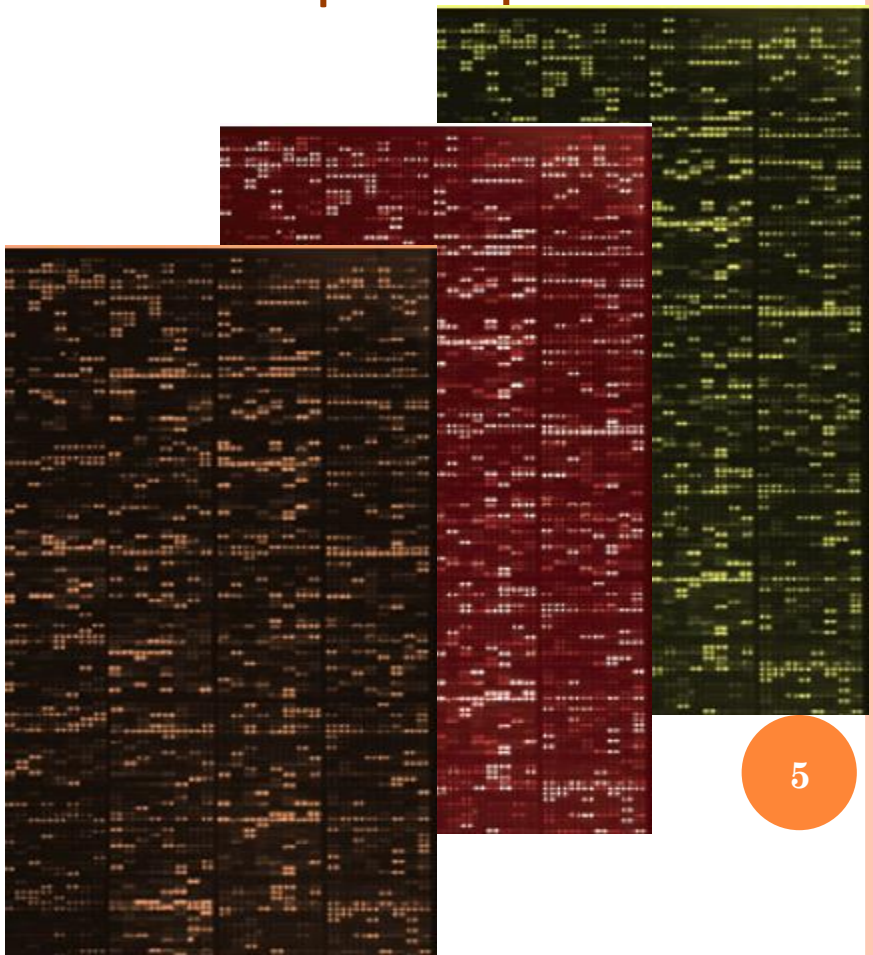
- Cellular processes and networks
- Investigation of protein-protein interactions
- Connection between the structure of proteins and their function
- Improve protein separation and protein profiling techniques

Protein profiling:

Accomplish Quantitative evaluation of protein levels

When proteins from one cell type are compared with those of another cell type shows us unique expression patterns at the protein level :

- ✓Diseased vs. Healthy
- ✓Treated vs. Untreated
- ✓Experimental vs. Control

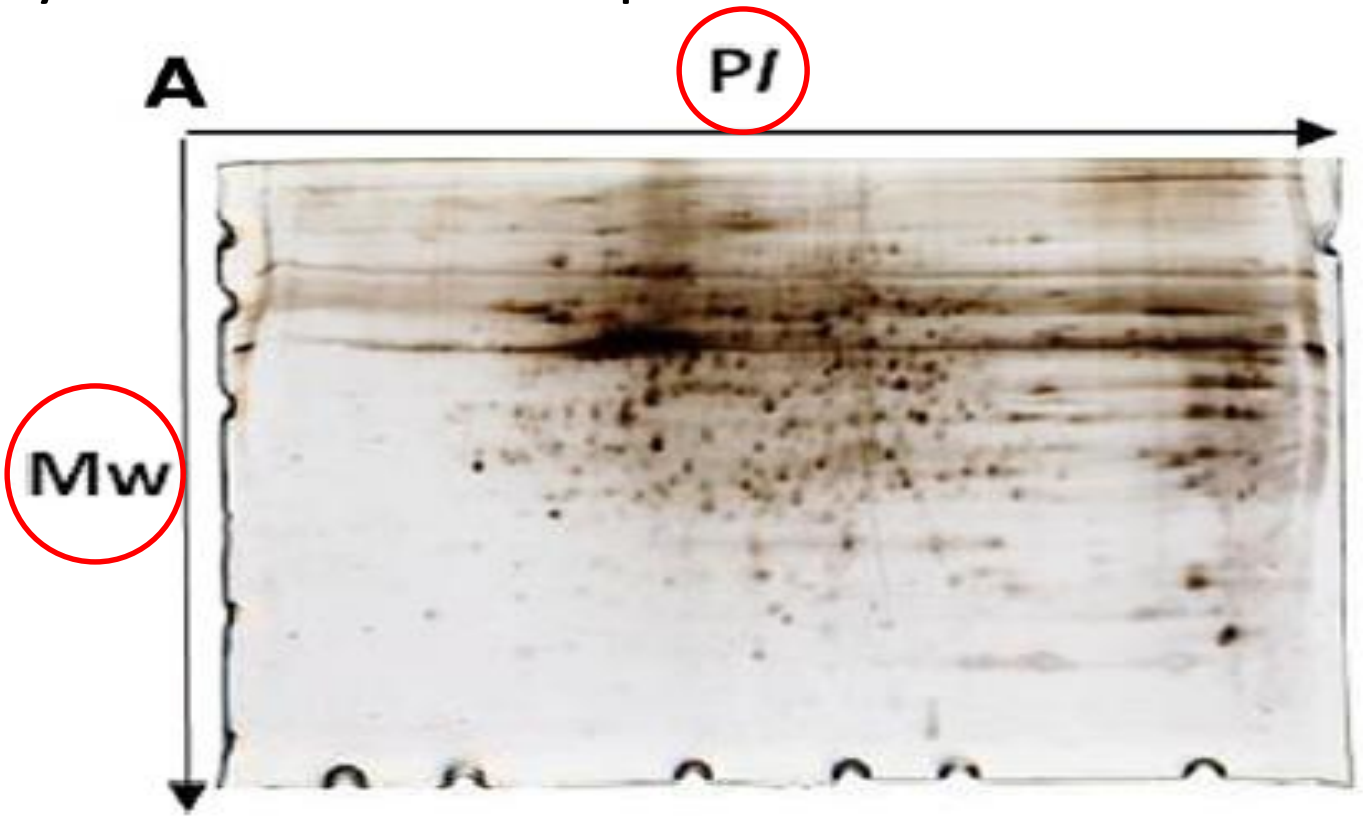


Two Dimensional Electrophoresis:

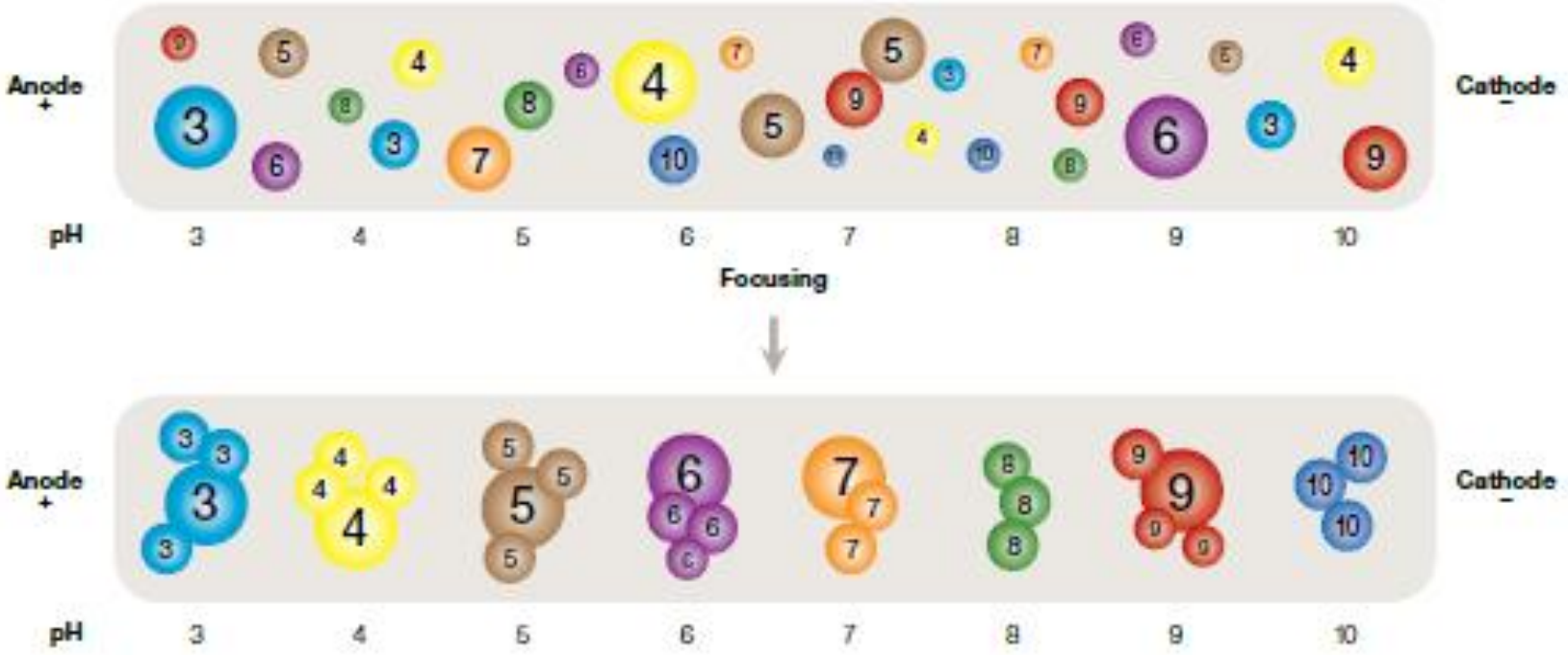
Is the classical method to separate proteins on the basis:

Charge ➲ Iso Electric Focusing

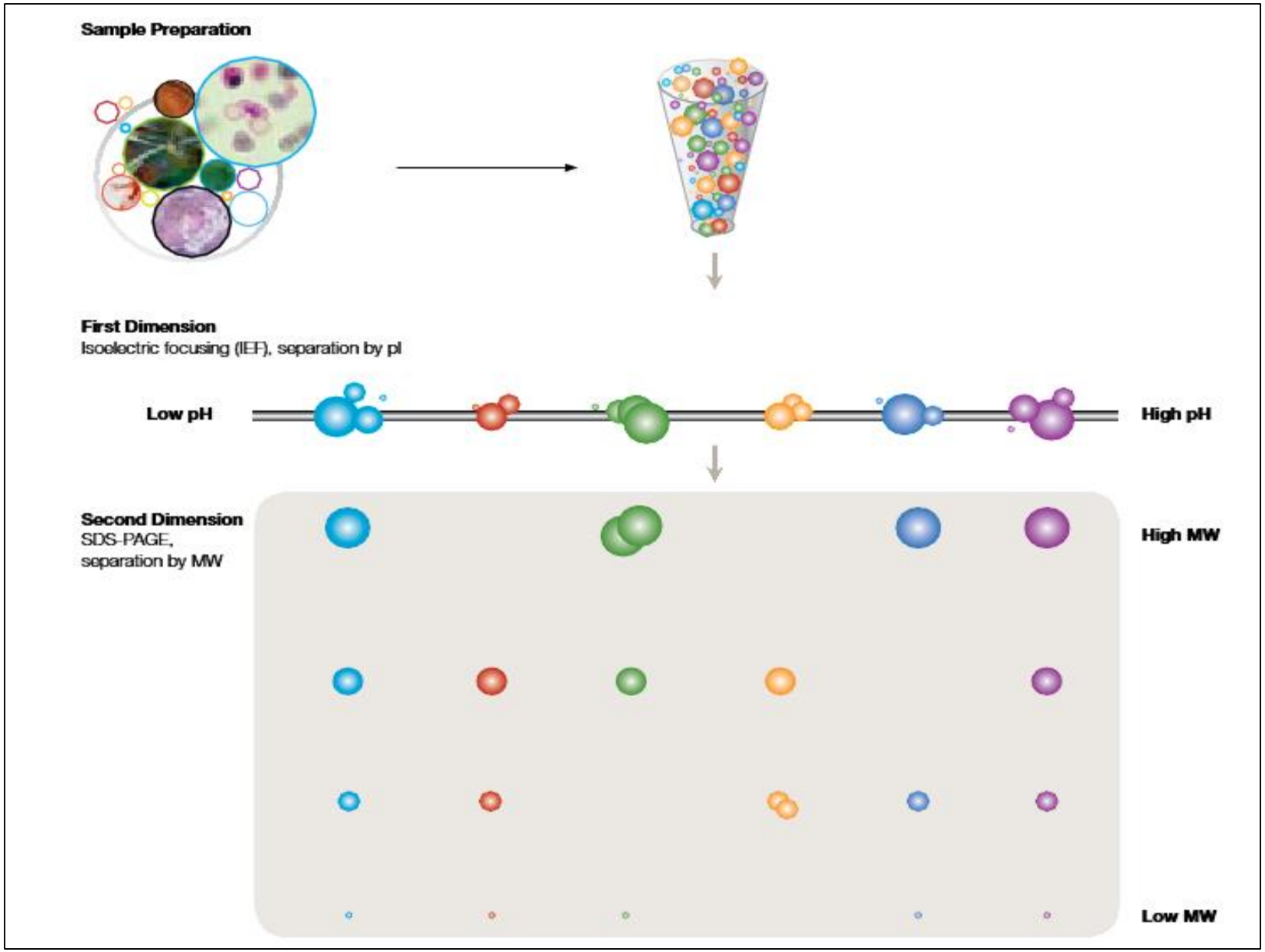
Molecular weight ➲ Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis



Iso Electric Focusing

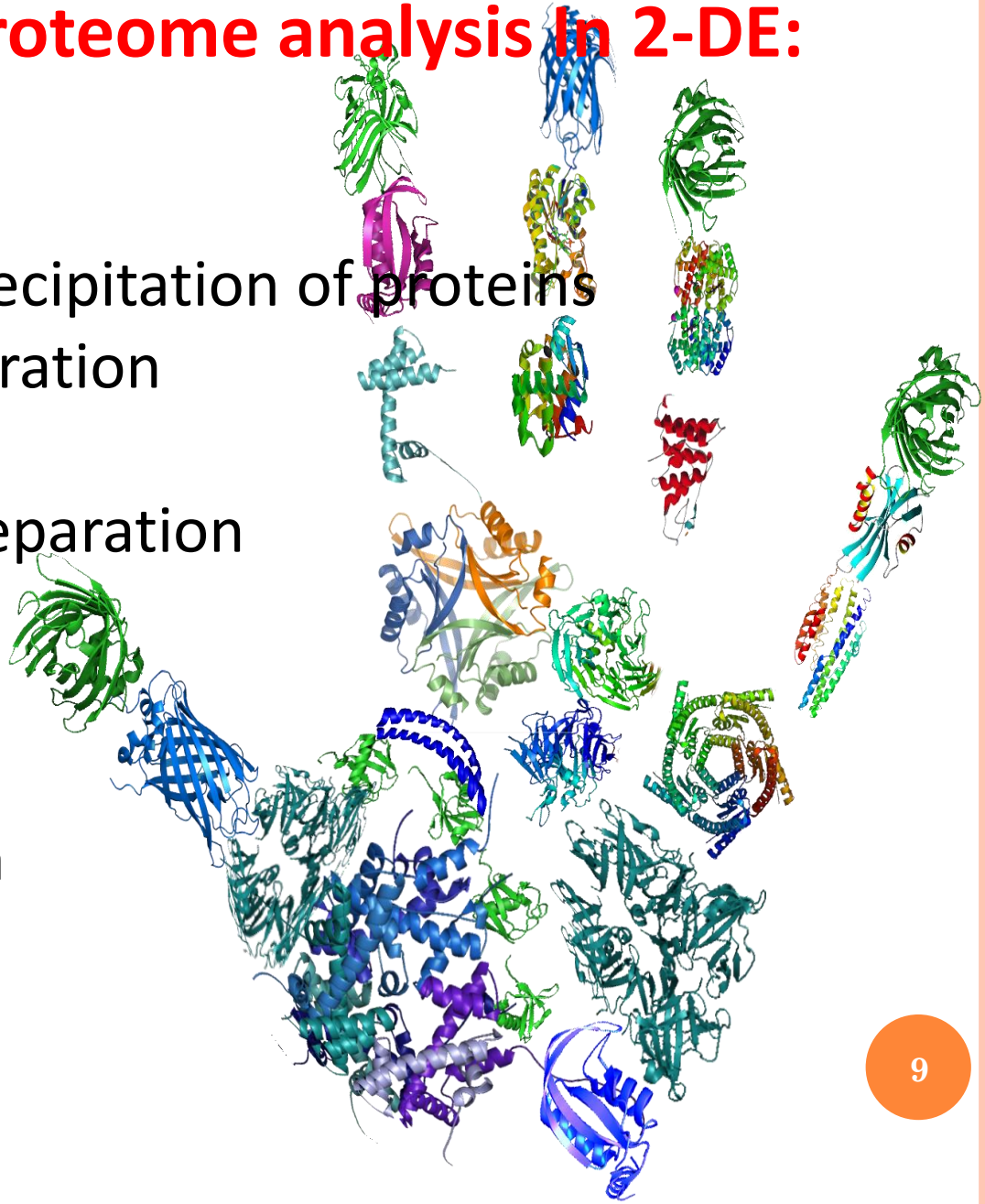


2DE:


Sample Preparation
First Dimension
Isoelectric focusing (IEF), separation by pI
Low pH
High pH
Second Dimension
SDS-PAGE,
separation by MW
High MW
Low MW

Challenges during proteome analysis In 2-DE:

1. Sample preparation
2. Solubilization and Precipitation of proteins
3. First-dimension separation
4. Equilibration
5. Second-dimension separation
6. Staining
7. Imaging
8. Image analysis
9. Protein identification



Applications and utilities of 2DE:

- Purity checks
- Drug discovery
- Cancer research
- Cell differentiation
- Bacterial pathogenesis
- Product characterization
- Whole proteome analysis
- Microscale protein purification
- Analysis of complex protein mixtures
- Detection of biomarkers and disease markers

Advantages and strengths of 2-DE:

☑ Compatible platform for further analysis
☑ Visualized mapping analysis
☑ Robustness

Limitations of 2-DE:

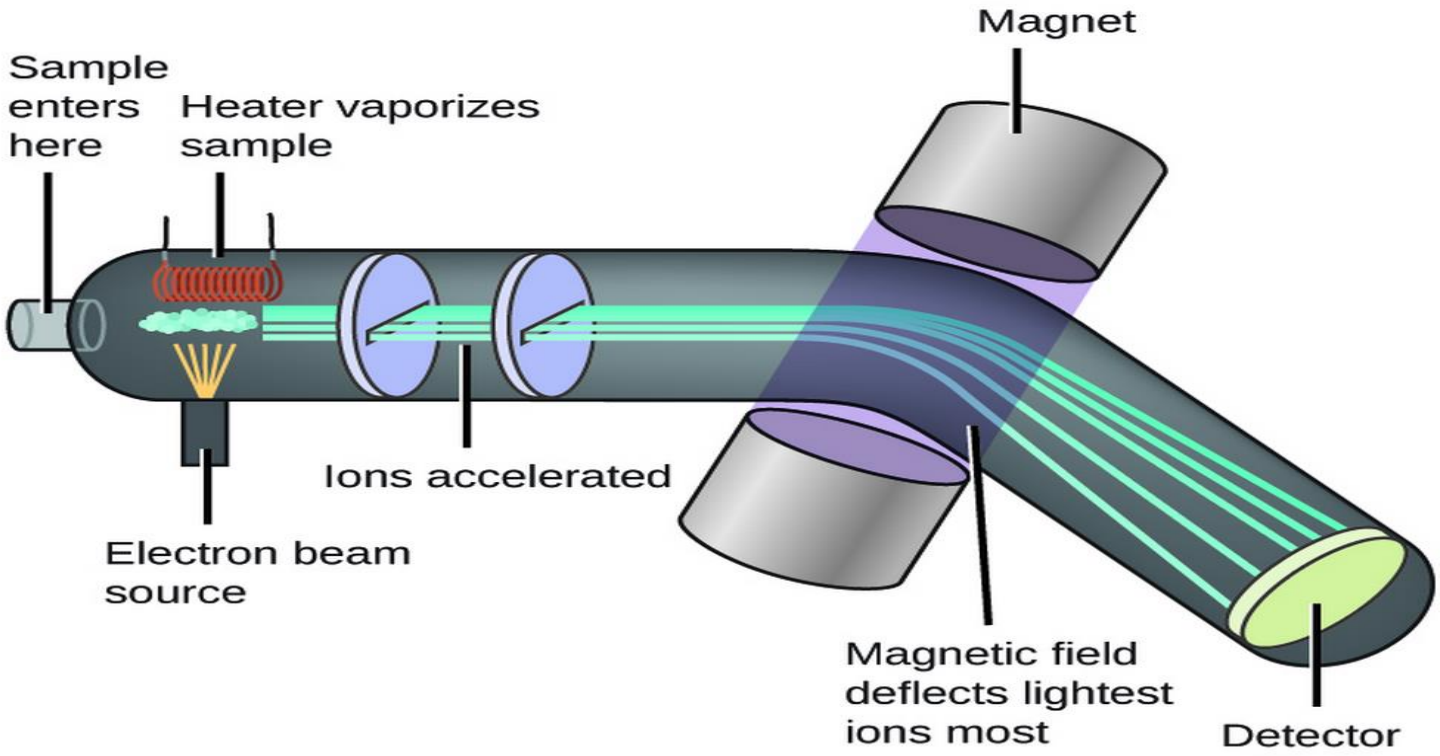
☒ Low reproducibility
☒ Narrow dynamic range of 2-DE
☒ Low throughput and labor- intensiveness
☒ Difficulty in separating hydrophobic and extremely acidic or basic proteins

Mass Spectrometry :

- ☑ Accuracy
- ☑ Selectivity
- ☑ High sensitivity
- ☑ High throughput capability

Some applications MS:

- ❖ Biomedical
- ❖ Sports' doping
- ❖ Forensic science
- ❖ Food authentication
- ❖ Pharmaceutical research



All instruments contain three main elements:

1. Ionization source
   - **M**atrix-**A**ssisted **L**aser **D**esorption/**I**onization **T**ime **O**f **F**light
   - **S**urface **E**nhanced **L**aser **D**esorption/**I**onization **T**ime **O**f **F**light
2. Mass analyzer
3. Detector

The first step in MS analysis:

Conversion of target analytes from the liquid or solid phase into gas phase ionized species

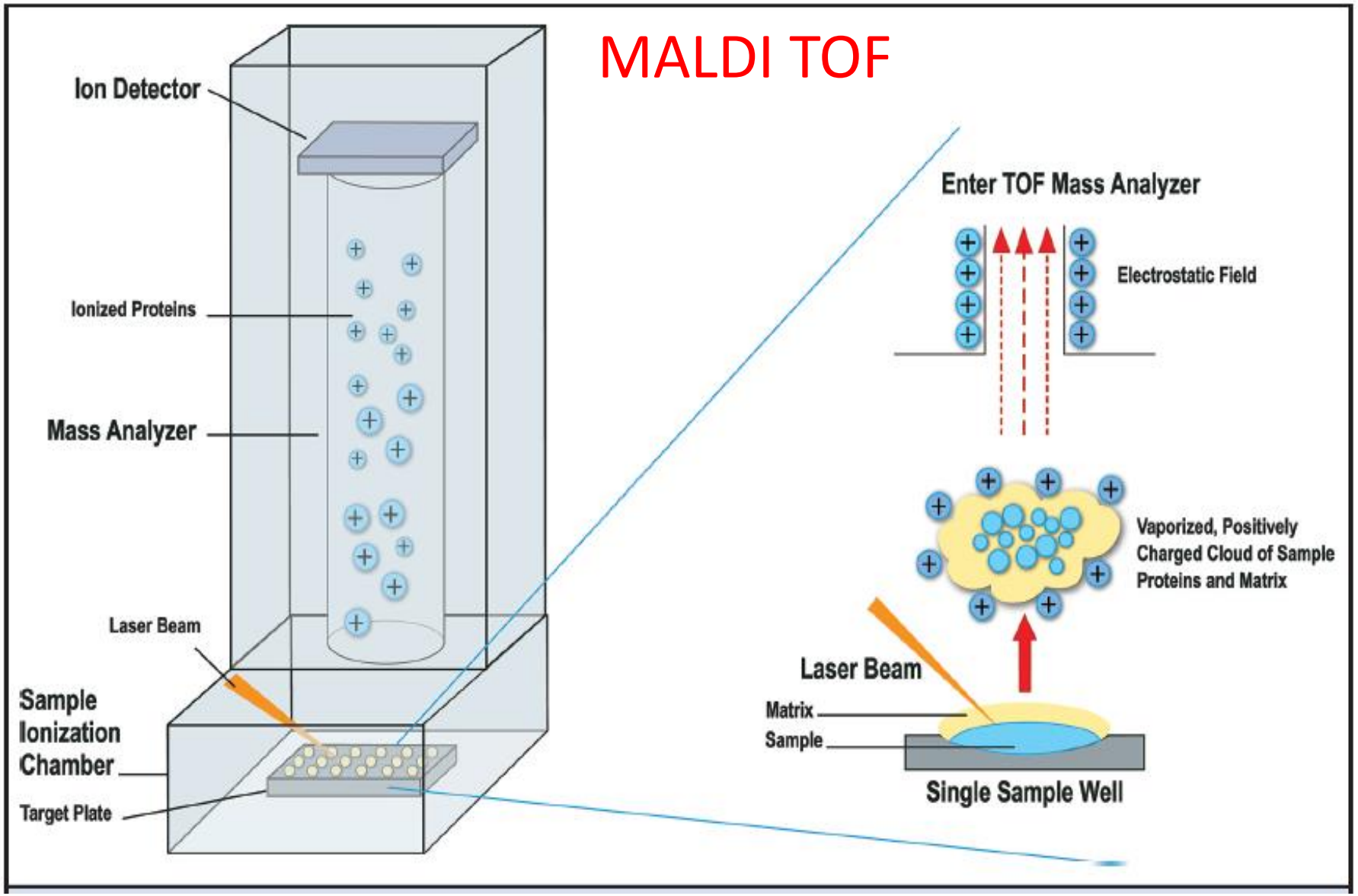
Mass-to-Charge ratio (*m/z*)

Steps of MALDI TOF:

1. Offline procedure of sample preparation
2. Application of the organic matrix and co-crystallization
3. Sample ionization in the MALDI source
4. Acceleration of generated ions in a TOF analyser
5. Detection of the ions based on their *m/z*
6. Generation of a mass spectrum

laser beam : Usually a nitrogen laser at a wavelength of 337 nm (UV)

MALDI TOF

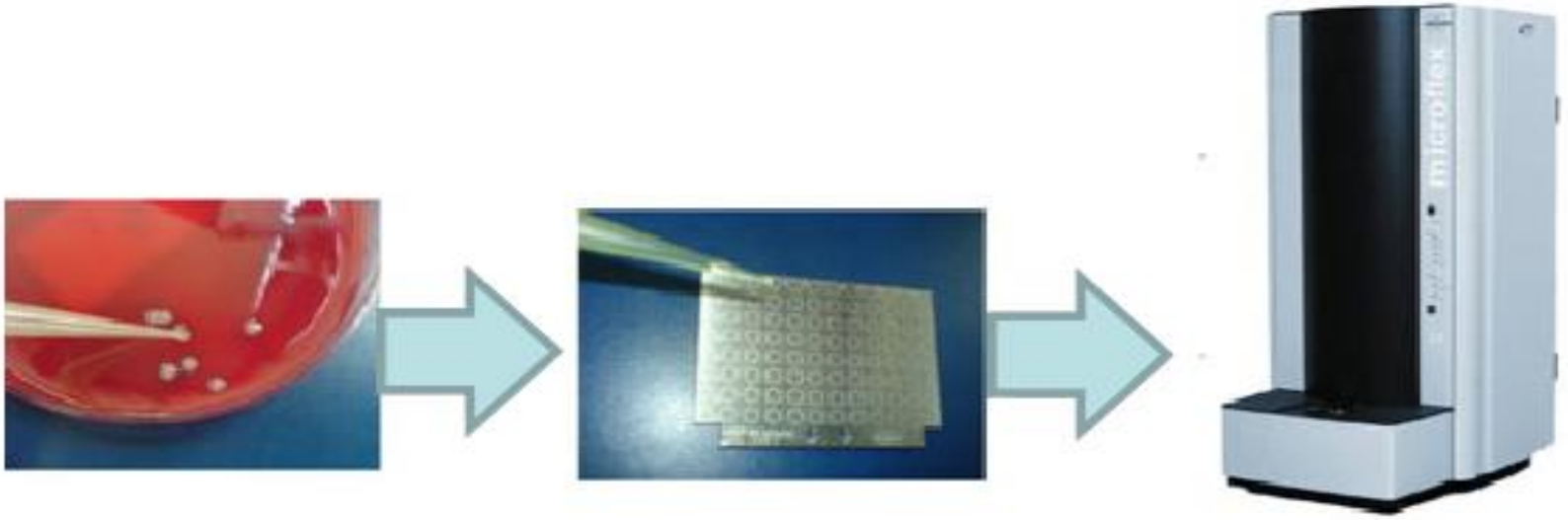

Ion Detector
Ionized Proteins
Mass Analyzer
Laser Beam
Sample Ionization Chamber
Target Plate
Enter TOF Mass Analyzer
Electrostatic Field
Vaporized, Positively Charged Cloud of Sample Proteins and Matrix
Laser Beam
Matrix
Sample
Single Sample Well

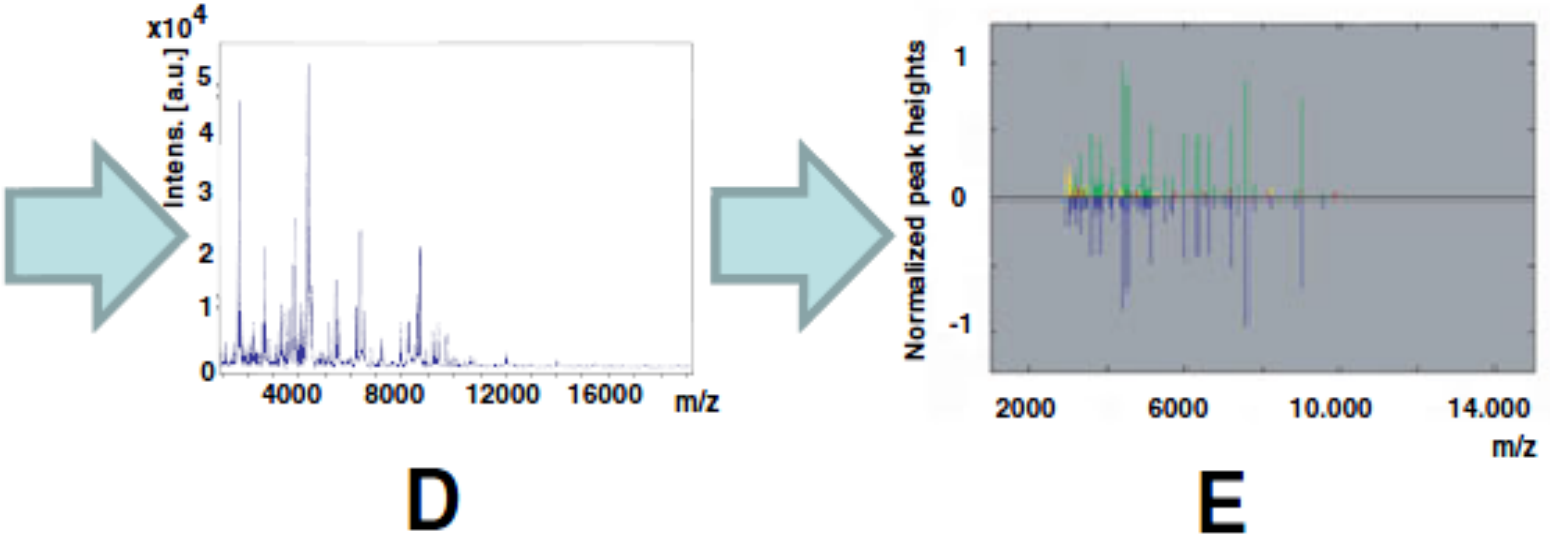
Critical steps in MALDI that affect the analysis:

- Type of matrix ⟶
  - ✓ Sinapinic acid
  - ✓ α-cyano-4-hydroxy cinnamic acid
- Laser parameters
- Technique of drying ⟶
  - ✓ Air
  - ✓ Vacuum
  - ✓ A stream of nitrogen gas

An interesting potential, Due to:

- Ease of use
- Speed of analysis
- Cost effectiveness

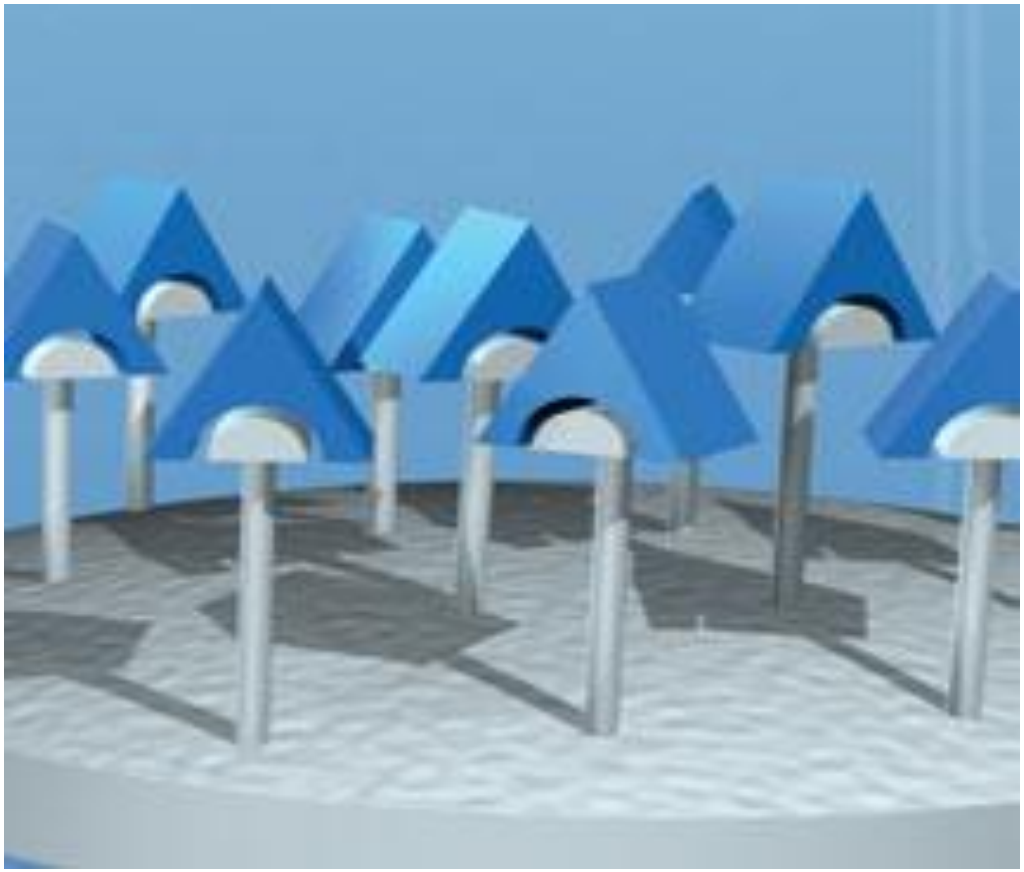

A
B
C


x10^4
Intens. [a.u.]
5
4
3
2
1
0
4000
8000
12000
16000
m/z
D
Normalized peak heights
1
0
-1
2000
6000
10.000
14.000
m/z
E

SELDI TOF:

Active role in the:

- ✓ Ionization
- ✓ Extraction
- ✓ Desorption
- ✓ Purification
- ✓ Modification
- ✓ Amplification

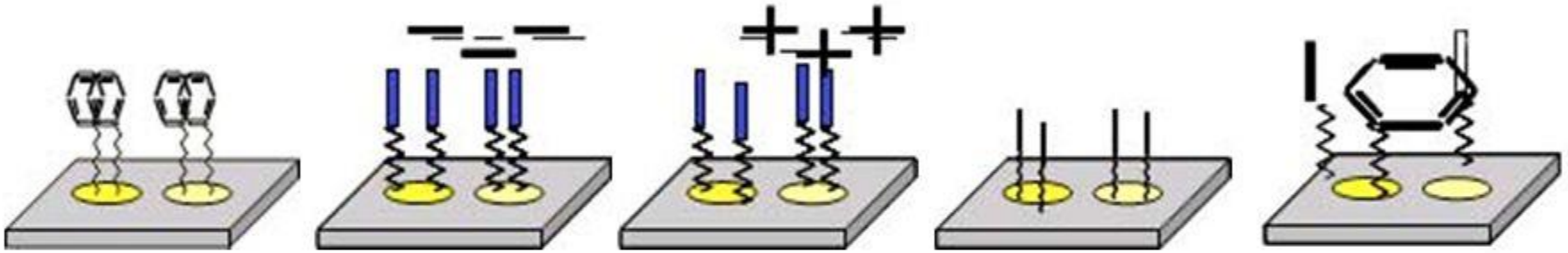


Three major components constitute SELDI-TOF:

- Protein Chip arrays
- Mass analyzer
- Data analysis software

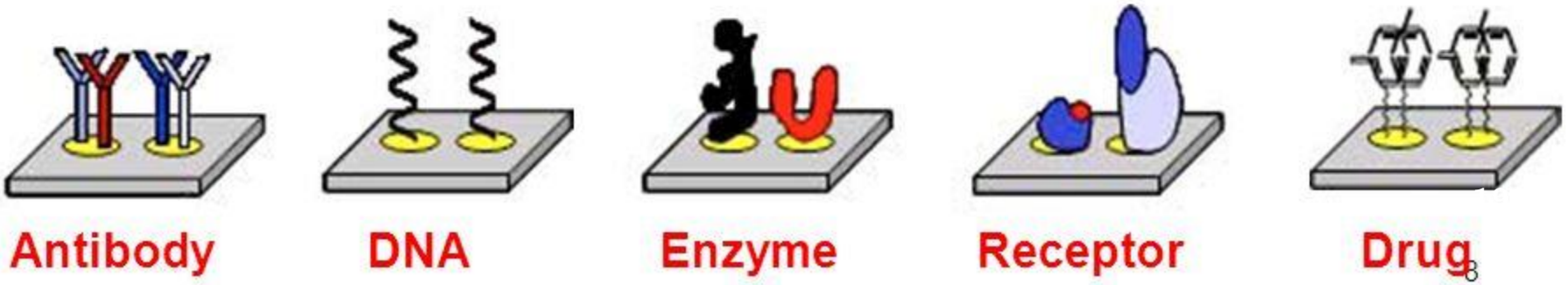
SELDI ProteinChip

- **Chemical Surfaces**

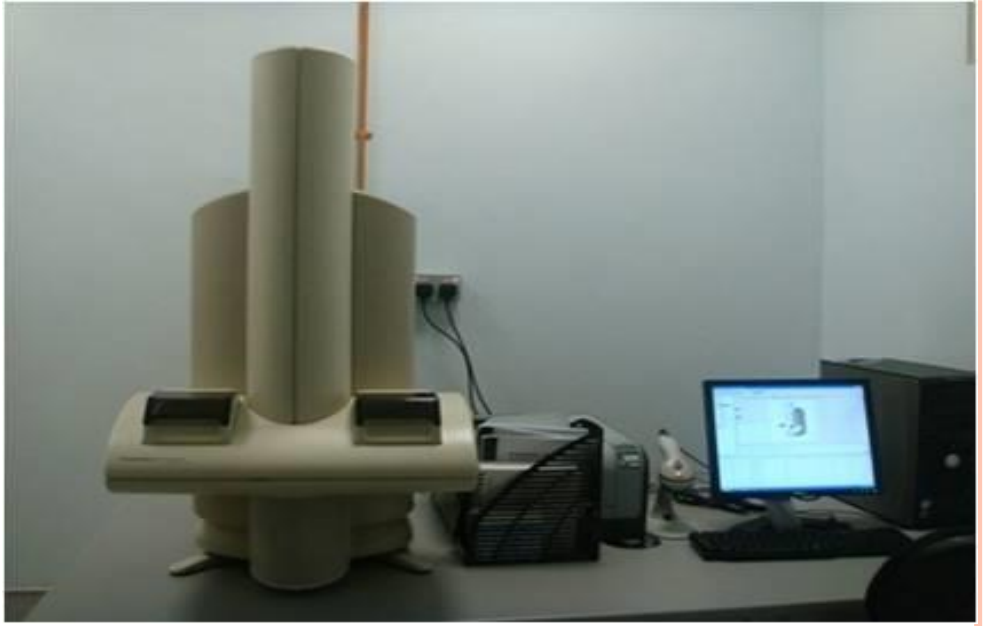


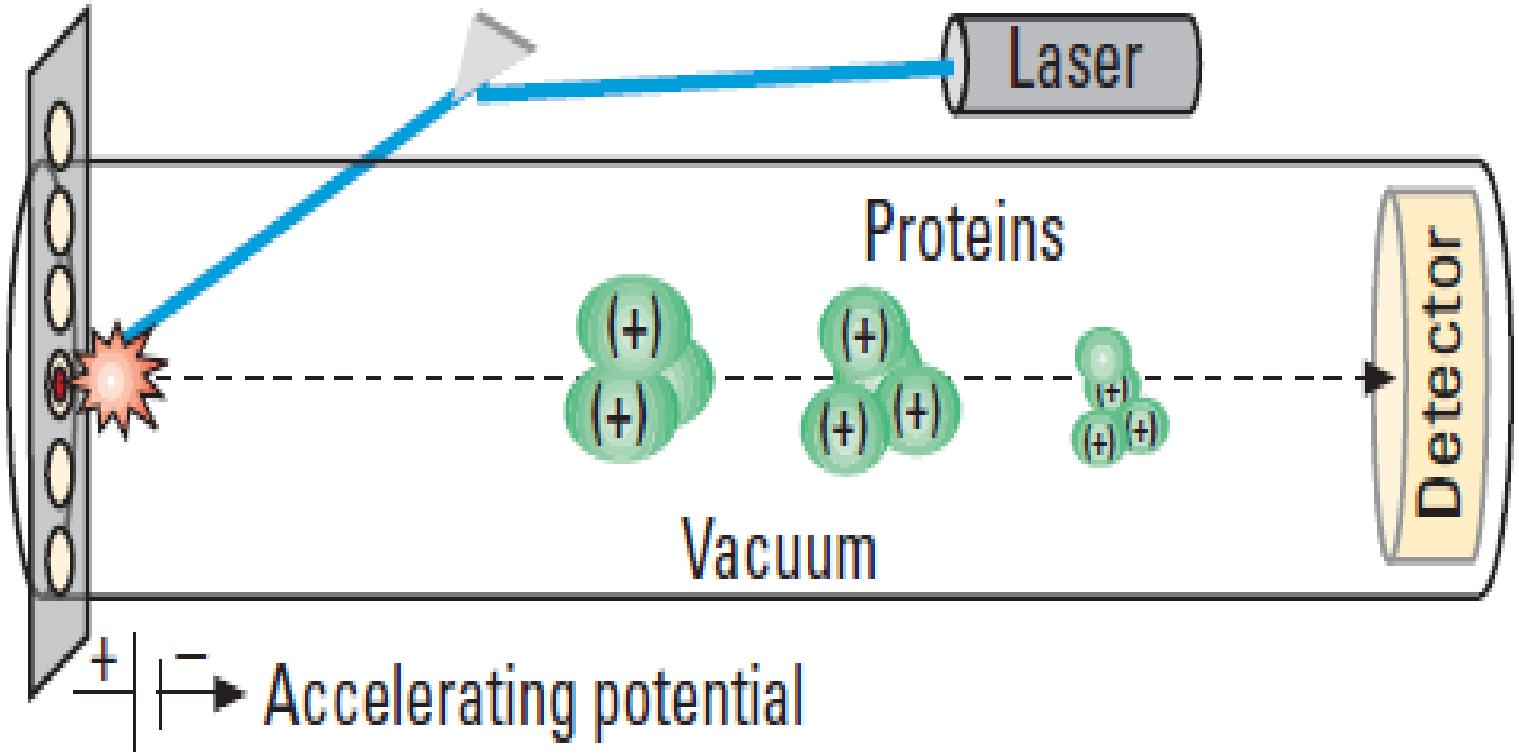
Immobilized Metal Affinity Capture

- **Biochemical Surfaces**



SELDI TOF:





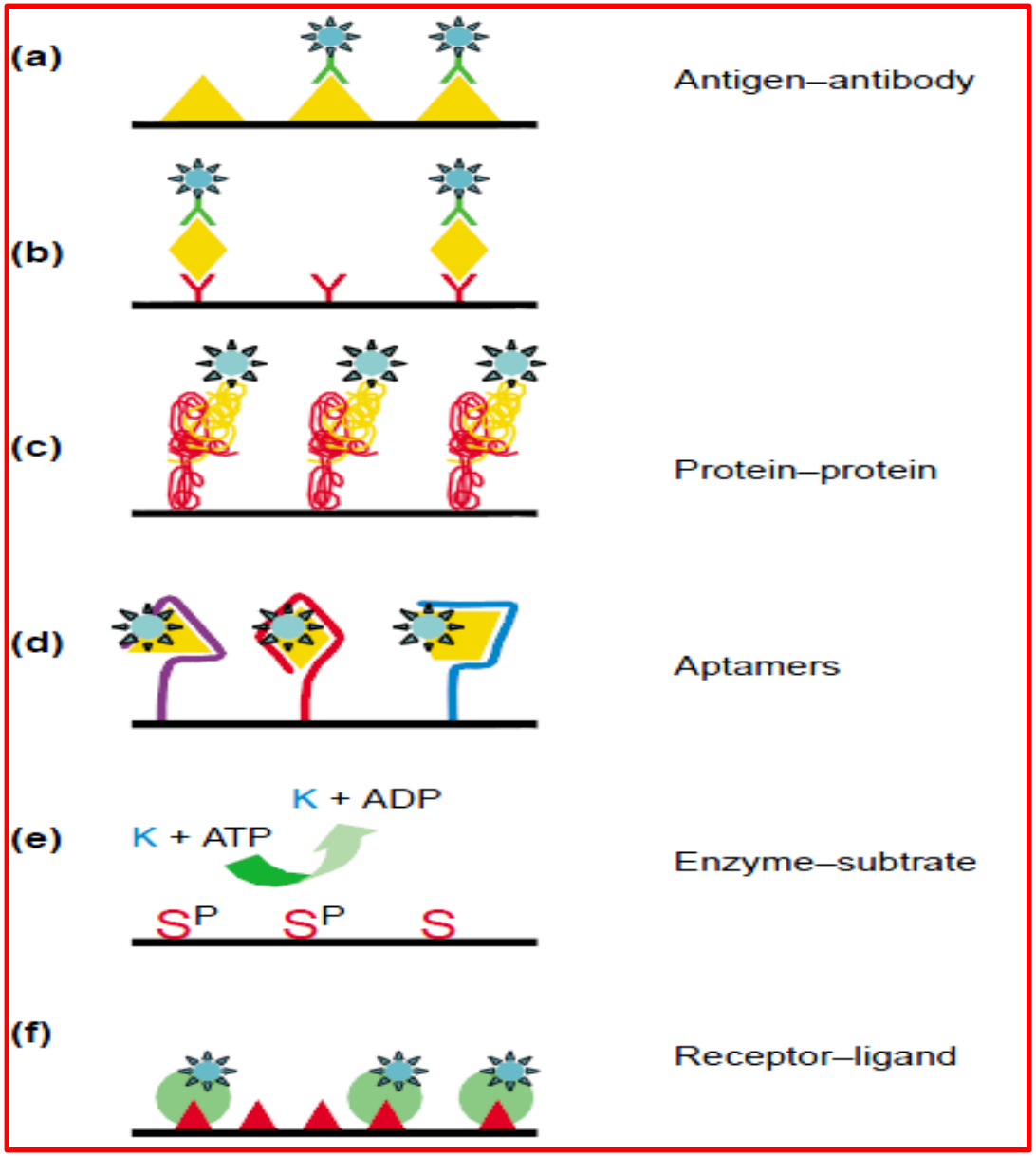
Types of protein microarrays

- Reverse phase microarrays
- Functional microarrays
- Analytical microarrays:
  - Specificities
  - Binding affinities
  - Protein expression levels of the proteins in the mixture

Protein microarray:

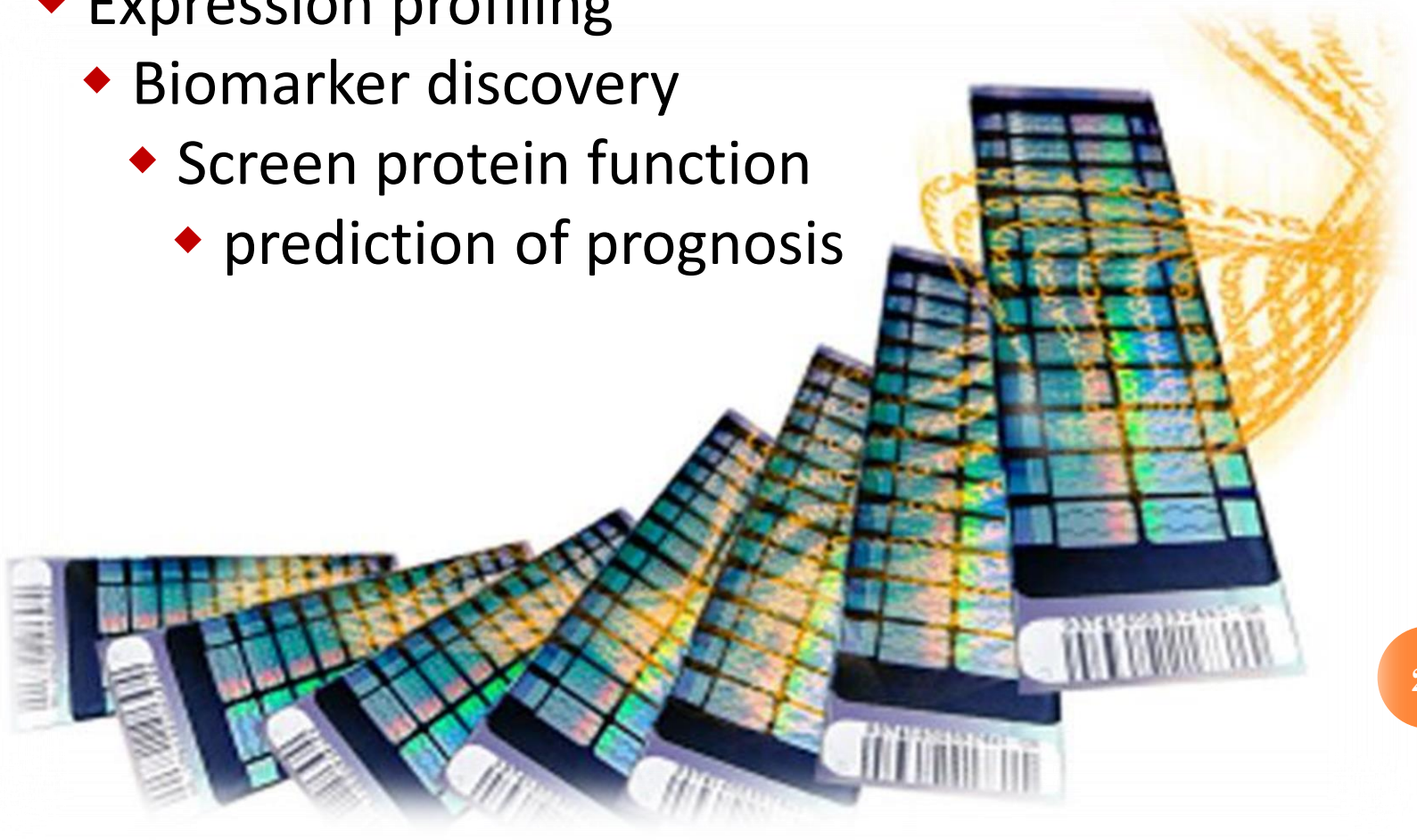
Proteins spotted in defined locations on a solid support are probed for interactions with a probe molecule

Classes of capture molecules for protein microarrays:



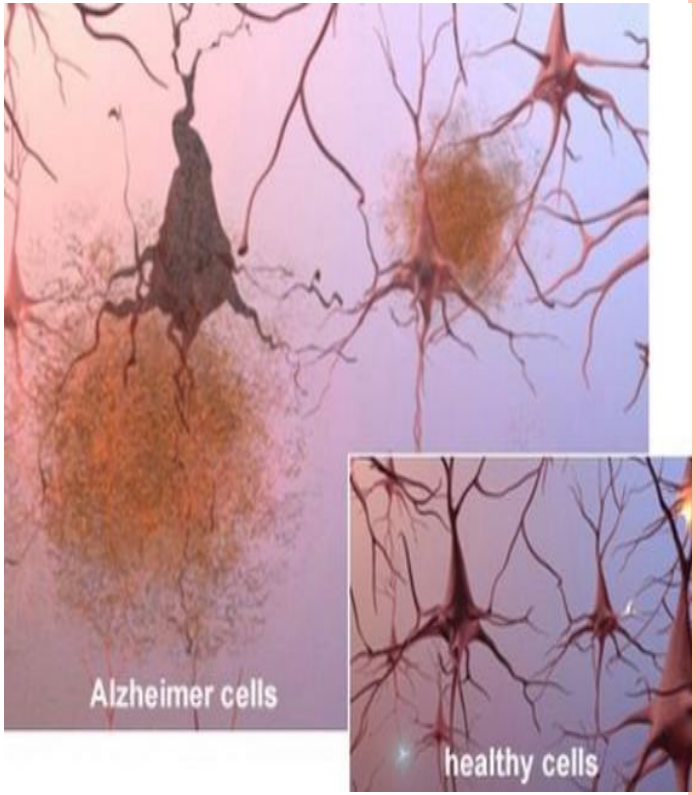
Protein array analysis is used to:

- Drug discovery
  - Clinical diagnosis
    - Antibody analysis
      - Expression profiling
        - Biomarker discovery
          - Screen protein function
            - prediction of prognosis



Beta amyloid peptide:

☞Major component of senile plaques deposited in the brains of individuals with Alzheimer's disease



✓A 39–42-residue peptide

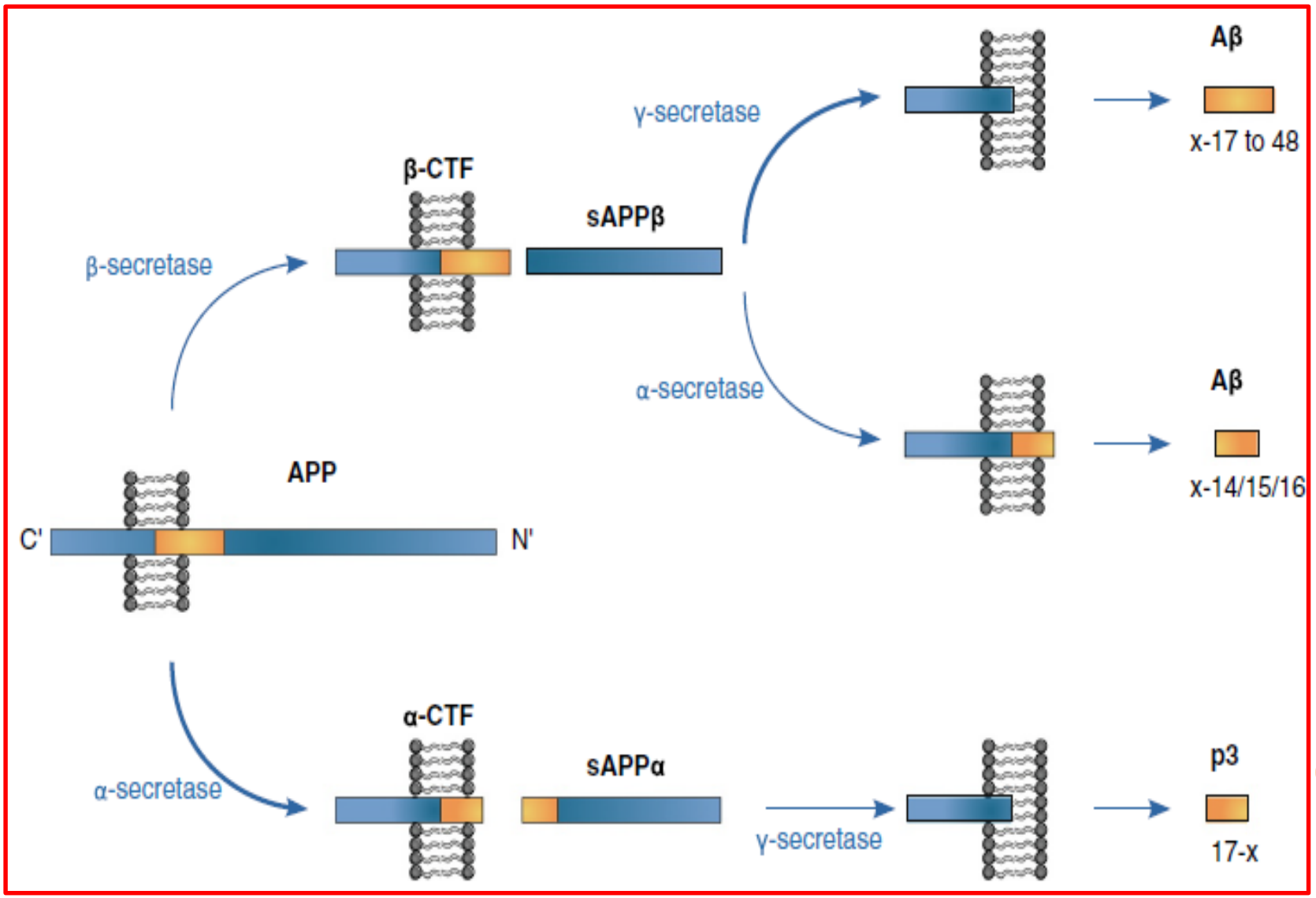
✓Amyloid Precursor Protein:

- Polypeptide chain of 695 or 770
- Trans membrane protein

The two best known forms :

Aβ1-40
Aβ1-42
} Depending on the number of amino acids in the peptide

Generation of different amyloid-beta domain derived peptides from the APP:



Journal of Alzheimer's Disease 47 (2015) 877–881
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IOS Press

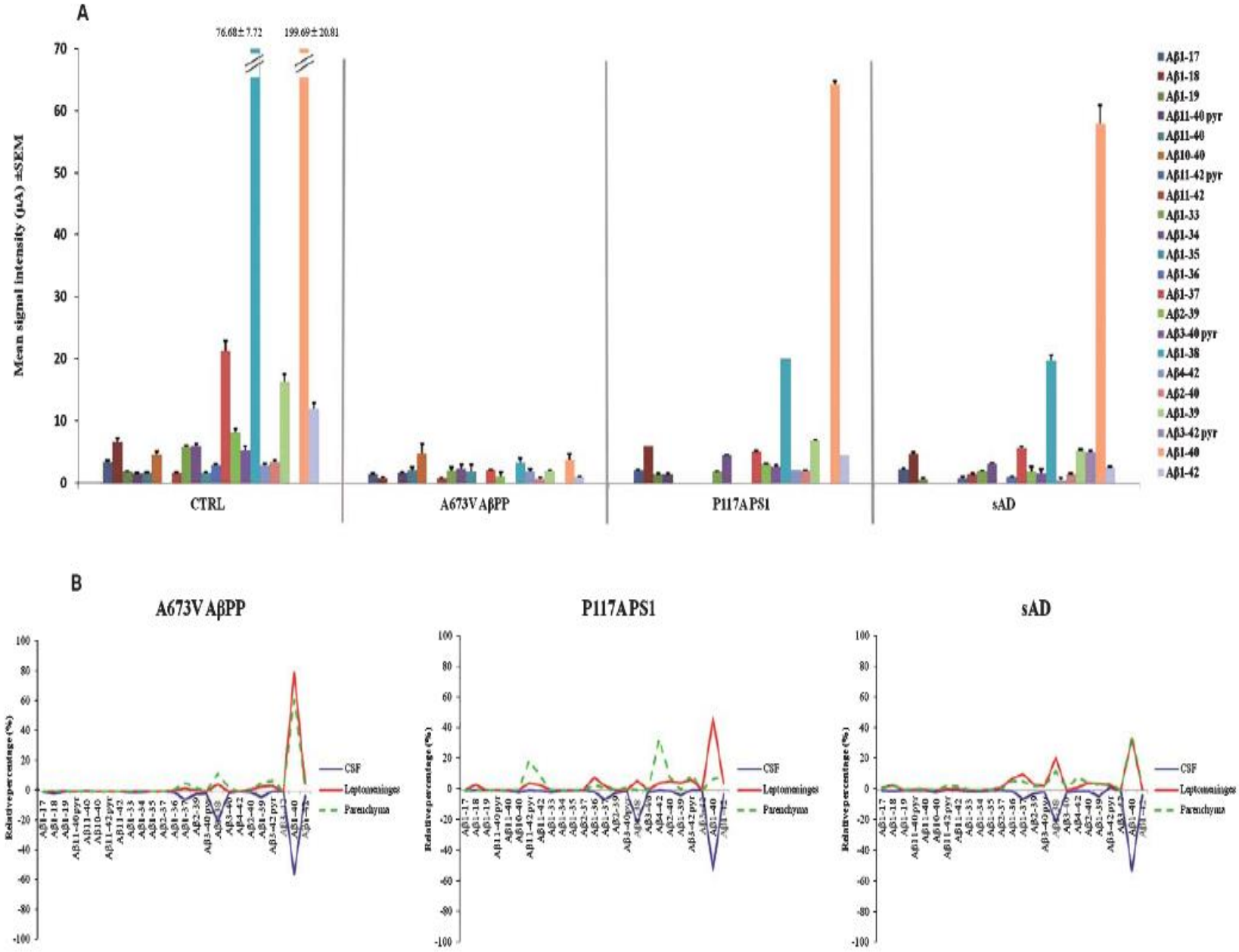
Short Communication

Mirror Image of the Amyloid-β Species in Cerebrospinal Fluid and Cerebral Amyloid in Alzheimer's Disease

Marcella Catania[a], Giuseppe Di Fede[a], Elisa Tonoli[b], Luisa Benussi[b], Claudio Pasquali[a], Giorgio Giaccone[a], Emanuela Maderna[a], Roberta Ghidoni[b] and Fabrizio Tagliavini[a,*]
[a]*Division of Neurology 5 and Neuropathology, IRCCS Foundation - Carlo Besta Neurological Institute, Milan, Italy*
[b]*Molecular Markers Laboratory, IRCCS Istituto Centro San Giovanni di Dio - Fatebenefratelli, Brescia, Italy*

Accepted 19 May 2015

Abstract. Alzheimer's disease (AD) is characterized by amyloid-β (Aβ) accumulation in brain that is paralleled by $A\beta_{1-42}$ reduction in cerebrospinal fluid (CSF). We analyzed the pattern of Aβ peptides, including the N- and C-terminal truncated fragments, in brain and CSF from two familial and one sporadic AD cases. We found that (i) each patient is characterized by a distinct Aβ profile in CSF and brain deposits and (ii) the CSF Aβ pattern mirrors the Aβ profile of cerebral amyloid. These results suggest the existence of different molecular AD subtypes which can be recognized by CSF analysis, enabling patient stratification.


A
76.68 ± 7.72
199.69 ± 20.81
Mean signal intensity (µA) ±SEM
CTRL
A673V AβPP
P117APS1
sAD
Aβ1-17
Aβ1-18
Aβ1-19
Aβ11-40 pyr
Aβ11-40
Aβ10-40
Aβ11-42 pyr
Aβ11-42
Aβ1-33
Aβ1-34
Aβ1-35
Aβ1-36
Aβ1-37
Aβ2-39
Aβ3-40 pyr
Aβ1-38
Aβ4-42
Aβ2-40
Aβ1-39
Aβ3-42 pyr
Aβ1-40
Aβ1-42
B
A673V AβPP
P117APS1
sAD
Relative percentage (%)
CSF
Leptomeninges
Parenchyma

Review

Pauline Bros, Vincent Delatour*, Jérôme Vialaret, Béatrice Lalere, Nicolas Barthelemy, Audrey Gabelle, Sylvain Lehmann* and Christophe Hirtz

Quantitative detection of amyloid-β peptides by mass spectrometry: state of the art and clinical applications

Abstract: Alzheimer's disease (AD) is the most common form of dementia in humans, and a major public health concern with 35 million of patients worldwide. Cerebrospinal fluid (CSF) biomarkers being early diagnostic indicators of AD, it is essential to use the most efficient analytical methods to detect and quantify them accurately. These biomarkers, and more specifically amyloid-β (Aβ) peptides, are measured in routine clinical practice using immunoassays. However, there are several limits to this immunodetection in terms of specificity and multiplexing of the multiple isoforms of the Aβ peptides. To overcome these issues, the quantification of these analytes by mass spectrometry (MS) represents an interesting alternative, and several assays have been described over the past years. This article reviews the different Aβ peptides quantitative MS-based approaches published so far, compares their pre-analytical phase, and the different quantitative

Detection of amyloid-β peptides

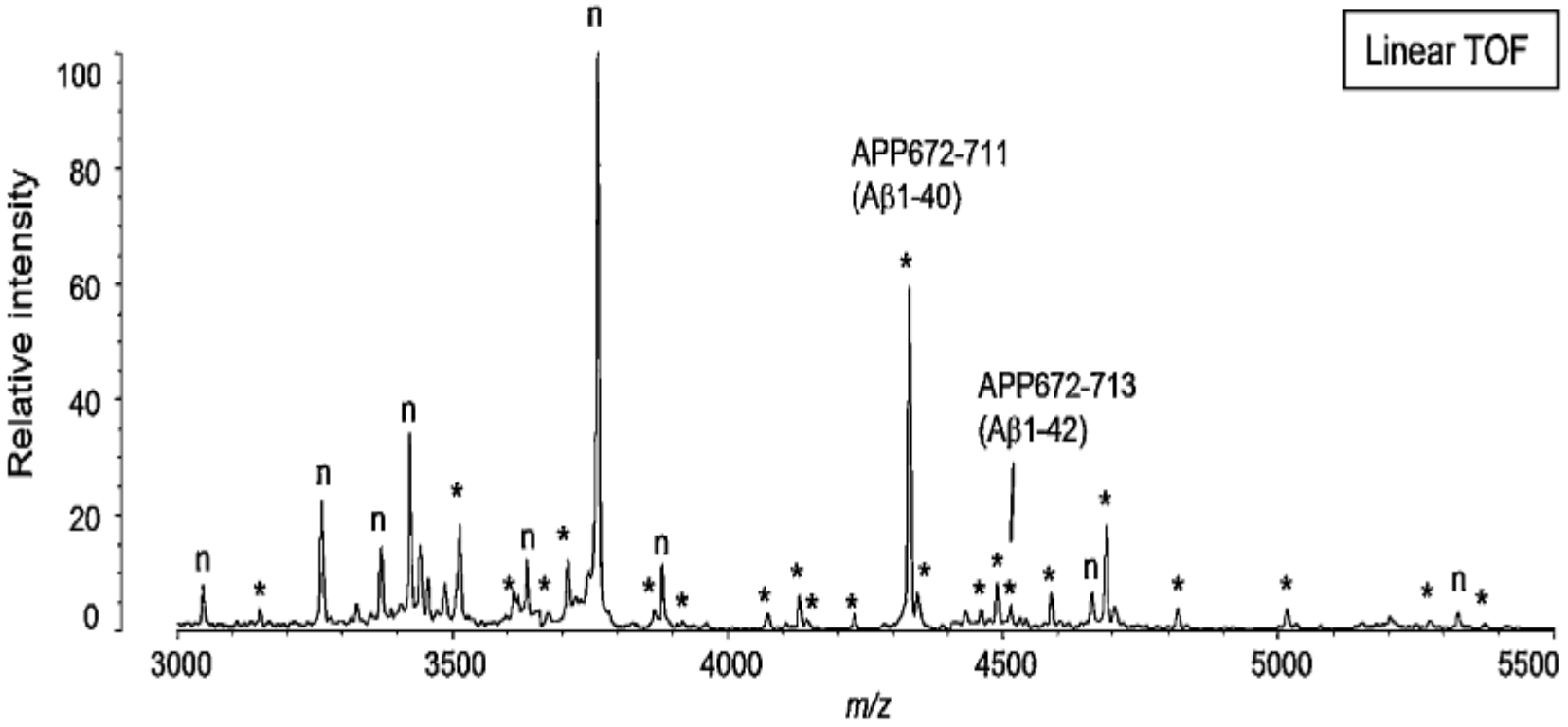
	ELISA	MALDI	SELDI	LC-MS/MS
1-38, 1-40, 1-42	X	X	X	X
1-13, 1-14, 1-15,1-16, 1-20, 1-28, 1-30, 1-37 ox, 1-38 ox, 1-39 ox, 1-40 ox, 1-42 ox, 1-43, 2-42, 3-40, 3-42, 5-40		X		X
1-17, 1-18, 1-19, 1-33, 1-34, 1-39		X	X	X
1-29, 2-15,2-16,2-17, 2-19, 2-38, 4-13, 4-14, 4-15, 4-16, 4-19				X
1-35, 1-36, 1-37, 1-40 x-x, 3-44, 3-47, 10-40, 11-40			X	
2-14			X	X
4-40, 4-42, 5-42, 7-42, 8-42, 9-40, 9-42, 10-42, pGlu 11-42, pGlu 3-40, pGlu 3-42		X		
11-42		X	X	

Identification and quantification of amyloid beta-related peptides in human plasma using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

By Naoki KANEKO,[*1,†] Rie YAMAMOTO,[*1] Taka-Aki SATO[*1,*2] and Koichi TANAKA[*1]

(Contributed by Koichi TANAKA, M.J.A.)

Abstract: Proteolytic processing of the amyloid precursor protein (APP) by β-secretase and γ-secretase leads to the generation and deposition of amyloid β (Aβ) in Alzheimer's disease (AD). N-terminally or C-terminally truncated Aβ variants have been found in human cerebrospinal fluid and cultured cell media using immunoprecipitation and mass spectrometry. Unfortunately, the profile of plasma Aβ variants has not been revealed due to the difficulty of isolating Aβ from plasma. We present here for the first time studies of Aβ and related peptides in human plasma. Twenty-two Aβ-related peptides including novel peptides truncated before the β-secretase site were detected in human plasma and 20 of the peptides were identified by tandem mass spectrometry. Using an internal standard, we developed a quantitative assay for the Aβ-related peptides and demonstrated plasma dilution linearity and the precision required for their quantitation. The present method should enhance the understanding of APP processing and clearance in AD progression.

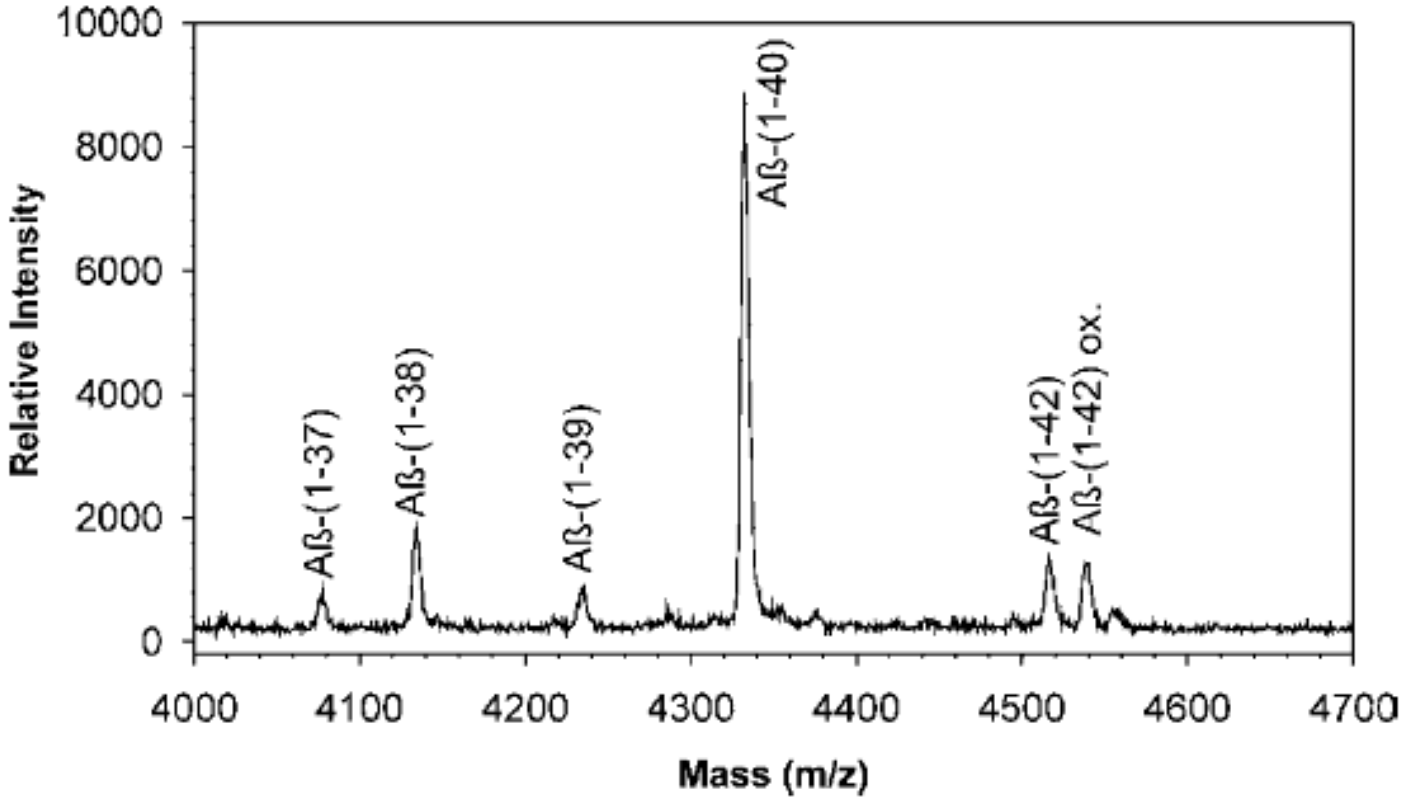

Linear TOF
APP672-711
(Aβ1-40)
APP672-713
(Aβ1-42)
Relative intensity
m/z
100
80
60
40
20
0
3000
3500
4000
4500
5000
5500

Molecular imaging of amyloid β peptides in mouse brain sections using mass spectrometry

Markus Stoeckli,* Dieter Staab, Matthias Staufenbiel, Karl-Heinz Wiederhold, and Luca Signor

Abstract

A method is presented for direct spatial analysis of amyloid β peptides in biological tissue sections. The technique takes advantage of the very high sensitivity of matrix-assisted laser desorption/ionization mass spectrometry and is implemented on a commercial instrument with modifications to only a few components and the software. With this setup, hundreds of molecular images can be generated simultaneously and within just a few minutes. The current features are an instrumental resolution of 50 μm and a sensitivity down to the attomol range. This new technology is applied to the study of amyloid β peptide distribution in brain sections from mice, showing features reminiscent of Alzheimer's disease.


Relative Intensity
10000
8000
6000
4000
2000
0
4000
4100
4200
4300
4400
4500
4600
4700
Mass (m/z)
Aß-(1-37)
Aß-(1-38)
Aß-(1-39)
Aß-(1-40)
Aß-(1-42)
Aß-(1-42) ox.

Thanks

References:

1.Şanlı Mohamed G, Turan T, Ekiz HA, Baran Y. The importance of protein profiling in the diagnosis and treatment of hematologic malignancies. 2011.
2 .Awad H, Khamis MM, El-Aneed AJASR. Mass spectrometry, review of the basics: ionization. 2015;50(2):158.٧٥-
3.Austen BM, Frears ER, Davies HJJopsaopotEPS. The use of Seldi ProteinChip™ Arrays to monitor production of Alzheimer's β-amyloid in transfected cells. 2000;6(9):459-69.
4.Gelfanova V, Higgs RE, Dean RA, Holtzman DM, Farlow MR, Siemers ER, et al. Quantitative analysis of amyloid-β peptides in cerebrospinal fluid using immunoprecipitation and MALDI-Tof mass spectrometry. 2007;6(2):149-58.